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Atty. Docket No.: 9409/2092

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Communi, et al.
Serial No.: 09/924,125
Filed: August 7, 2001
Entitled: NATURAL LIGAND FOR ORPHAN
G. PROTEIN COUPLED RECEPTOR
GPR86 AND METHODS OF USE

Examiner: Not Assigned

Group Art Unit: 1614

Conf. No.: 3058

Box: Missing Parts
U.S. Patent and Trademark Office
P.O. Box 2327
Arlington, Virginia 22202

AMENDMENT

Sir:

This paper is submitted in response to the Notice to File Missing Parts mailed by the USPTO on 08/30/2001.

In the Specification:

1. On page 17, please replace the paragraph extending from lines 6-7 with the following replacement paragraph:

Figure 1 represents nucleotide (SEQ ID NO: 1) and deduced amino acid sequence (SEQ ID NO: 2) of the human GPR86 (P2Y₁₃) receptor according to the invention.

2. On page 41 of the specification, please replace the paragraph from lines 4 – 18 with the following replacement paragraph:

The NF- κ B binding element has the consensus sequence GGGGACTTTCC (SEQ ID NO: 3). A large number of genes have been identified as NF- κ B responsive, and their control elements can be linked to a reporter gene to monitor GPCR activity. A small sample of the genes responsive to NF- κ B includes those encoding IL-1 β (Hiscott et al., 1993, Mol. Cell. Biol. 13: 6231-6240), TNF- α (Shakhov et al., 1990, J. Exp. Med. 171: 35-47), CCR5 (Liu et al., 1998, AIDS Res. Hum. Retroviruses 14: 1509-1519), P-selection (Pan & McEver, 1995, J. Biol. Chem. 270: 23077-23083), Fas ligand (Matsui et al., 1998, J. Immunol. 161: 3469-3473), GM-CSF (Schreck & Baeuerle, 1990, Mol. Cell. Biol. 10: 1281-1286) and I κ B α (Haskill et al., 1991, Cell 65: 1281-1289). Each of these references is incorporated herein by reference. Vectors encoding NF- κ B-responsive reporters are also known in the art or can be readily made by one of skill in the art using, for example, synthetic NF- κ B elements and a minimal promoter, or using the NF-

κ B-responsive sequences of a gene known to be subject to NF- κ B regulation. Further, NF- κ B responsive reporter constructs are commercially available from, for example, CLONTECH.

3. On page 58 of the specification, please replace the paragraph extending from lines 16 – 26 with the following replacement paragraph:

Specific oligonucleotide primers were synthesized on the basis of the sequence of the GPR86 human receptor: a sense primer 5'-CCGGAATTCACCATGAACACCACAGTGATGC-3' (SEQ ID NO: 4) and an antisense primer 5'-CTTGTCTAGATCAGCCTAAGGTTATGTTGTC-3' (SEQ ID NO: 5). A polymerase chain reaction (PCR) was performed on three different spleen cDNAs using the Platinum Pfx DNA Polymerase. The amplification conditions were as follows: 94°C, 15 s; 50°C, 30 s; 68°C, 2 min for 35 cycles. Amplifications resulted in a fragment of 1 kilobase containing the entire coding sequence of the GPR86 gene. The coding sequence was then subcloned between the EcoRI and XbaI sites of the bicistronic pEFIN5 expression vector and sequenced on both strands for each of the three cDNAs using the BigDye Terminator cycle sequencing kit (Applied Biosystems, Warrington, Great Britain).

4. Please replace the paragraph on page 59 from lines 25- 33 and on page 60 from lines 1-5 with the following replacement paragraph:

Reverse transcription-polymerase chain reaction (RT-PCR) experiments were carried out using a panel of polyA⁺ RNA (Clontech). The GPR86 primers were as follows: GPR86 sense primer (5'-TGTGTCGTTTTCTTCGGTG-3') (SEQ ID NO: 6) and GPR86 antisense primer (5'-CTGCCAAAAGAGAGTTG-3') (SEQ ID NO: 7). The expected size of the amplified DNA band was 575 bp. Two primers synthesized on the basis of aldolase coding sequence were used as controls to produce a product with an expected size of 443 bp: aldolase sense primer 5'-GGCAAGGGCATCCTGGCTGC-3' (SEQ ID NO: 8) and aldolase antisense reverse 5'-TAACGGGCCAGAACATTGGCATT-3' (SEQ ID NO: 9). Approximately 75 ng of poly A⁺ RNA was reverse transcribed with Superscript II (Life Technologies, Inc., Merelbeke, Belgium) and used for PCR. PCR was performed using the Taq polymerase under the following conditions: denaturation at 94°C for 3 min, 38 cycles at 94°C for 1 min, 58°C for 2 min and 72°C for 2 min. Aliquots (10 μ l) of the PCR reaction were analysed by 1% agarose gel electrophoresis.

REMARKS

Applicants submit that herewith is a sequence listing in compliance with 37 C.F.R. §1.821-1.825, including an initial paper copy and a computer readable form as required by the Notice to File Missing Parts. Applicants request that the above amendments be incorporated into the said application. A separate sheet is attached which shows the changes made to the original paragraphs amended herein.

The amendments to the specification are made solely to add sequence identifiers referring to the sequences listed in the sequence listing. The amendments add no new matter. In accordance with 37 C.F.R. §1.821 (f), applicants hereby state that the paper copy and the computer readable form of the Sequence Listing submitted herewith in the above-identified patent application are supported in the application and contain no new matter. Applicants further

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state that the information recorded in computer readable form is identical to the information in the paper copy.

Date:

12/5/01



Name: Kathleen M. Williams

Registration No.: 34,380

Palmer & Dodge LLP

111 Huntington Avenue

Boston, MA 02108

Tel: 617-~~239~~-0100



Marked-up Version of Amendments:

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